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[Claim(s)]

[Claim 1] The fixed method to the solid phase carrier of the DNA fragment characterized by forming a covalent bond between this compound and a DNA fragment by performing optical radiation, contacting a DNA fragment to the solid phase carrier which the compound in which activity is shown by optical radiation has combined with the surface in the liquid phase.

[Claim 2] The fixed method to the solid phase carrier of the DNA fragment according to claim 1 characterized by the compound in which activity is shown by optical radiation being a compound which has nitrene, carbene, a radical, a carbon electrophilic agent, a diazonium machine, an azido group, a JIAJIRIN ring, or a diazo group.

[Claim 3] The DNA chip obtained by Claim 1 or a method given in 2.

[Claim 4] The process which gives the water liquid containing the nucleic acid fragment sample which carried out the sign with the fluorescent substance or the radioactive substance to the surface of a DNA chip according to claim 3, The process which fixes the DNA fragment currently fixed to the DNA chip, and the nucleic acid fragment sample which has complementation on a DNA chip by hybridization, And the detection method of a nucleic acid fragment which consists of a process which detects fluorescent labeling or the radioactive sign of a sign nucleic acid fragment sample fixed on the DNA chip of having complementation to the DNA fragment on a DNA chip.

[Claim 5] The process which gives the water liquid containing the intercalator which has a fluorescence generating machine or a conductive machine, and a nucleic acid fragment sample to the surface of a DNA chip according to claim 3, The process which fixes the DNA fragment currently fixed to the DNA chip, and the nucleic acid fragment sample which has complementation on a DNA chip by hybridization, And consist of a process which detects the electric current which flows through the fluorescence or the conductive machine generated from the fluorescence generating machine of the intercalator incorporated in the hybrid construction formed from the DNA fragment and nucleic acid fragment sample of the DNA chip. The detection method of a nucleic acid fragment of having complementation to the DNA fragment on a DNA chip.

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the fixed method on the surface of a solid phase carrier of a DNA fragment required for production of the high-density array (DNA chip) which aligned many DNA fragments and oligonucleotides very useful to simultaneous analyses, such as gene expression, variation, and polymorphism, in the solid phase surface. This invention relates also to the detection method of a nucleic acid fragment of having complementation again to the DNA fragment on the DNA chip manufactured by the fixed method on the surface of a solid phase carrier of the DNA fragment, and a DNA chip.

[0002]

[Description of the Prior Art] The technical development for analyzing efficiently all the gene functions of a variegated living thing is progressing, and the DNA chip is used as the analysis means. A DNA chip is usually in the form of the micro array which carried out alignment fixation of many DNA fragments at solid phase carriers, such as slide glass. A DNA fragment sample with the DNA fragment currently fixed to the DNA chip and complementation is fixed on a DNA chip by hybridization, and it is used for the method of detecting. The method of using an intercalator with the method of using fluorescent labeling or the radioactive sign beforehand combined with the DNA fragment sample as a detection means of the formed hybrid and the fluorescence generating machine incorporated into a hybrid, or a conductive machine etc. is known.

[0003] The DNA chip technology using a DNA chip can be applied also to biological molecules other than DNA, and is expected as what provides research and development of innovative drug development research, diagnosis of the illness and development of a prevention method, energy, the measure against an environmental problem, etc., etc. with a new means.

[0004] [that use of the DNA chip as an analysis means of DNA has taken shape] The base sequence of DNA [with hybridization with an oligonucleotide] It starts for the method (SBH, sequencing by hybridization) of determining to have been devised (Drmanac, R.et al., Genomics, 4, page 114 (1989)). Although SBH was the way the limit of the nucleotide sequence which uses gel electrophoresis was conquerable, it did not result in utilization.

[0005] Then, DNA chip production technology is developed and investigate gene expression, variation, polymorphism, etc. efficiently for a short time. What is called HTS (high throughput screening) became possible (Fodor, S.P.A., Science, 251, page 767 (1991) and Schena, M., Science, 270, page 467 (1995)).

[0006] However, in order to put DNA chip use technology in practical use, the production technology of the DNA chip for making the solid phase carrier surface carry out alignment fixation of many DNA fragments and oligonucleotides is needed.

[0007] As the production method of a DNA chip, the method (it is called the "on-tipping method".) of compounding a direct DNA fragment on the solid phase carrier surface and the method of fixing to the solid phase carrier surface the DNA fragment prepared independently beforehand are known. The method (it is called "masking technology".) of performing alternative synthesis in the predetermined field of a minute matrix as an on-tipping method combining use of the blocking group removed alternatively, and the photo lithography technology and solid phase synthesis technology used for semiconductor manufacture by optical radiation is typical.

[0008] As a method of fixing to the solid phase carrier surface the DNA fragment prepared beforehand, there

is the following method according to the kind of DNA fragment, or the kind of solid phase carrier.
(1) The DNA fragment to fix [in the case of cDNA (complementary DNA which used mRNA as the mold and compounded it), or a PCR product (DNA fragment which made cDNA amplify by the PCR method)]
Generally the method of carrying out point arrival to the solid phase carrier surface which carried out the surface treatment of these with Pori cations (poly ricin, polyethyleneimine, etc.) using the spotter equipment with which DNA chip production equipment was equipped, and carrying out an electrovalent bond to a solid phase carrier using the electric charge of DNA is used. Moreover, the method of using the silane coupling agent which has an amino group, an aldehyde group, an epoxy group, etc. as the processing method on the surface of a solid phase carrier is also used (Geo, Z.et al., Nucleic Acid Research, 22, 5456-5465 (1994)). In this case, since an amino group, an aldehyde group, etc. are introduced into the solid phase carrier surface by the covalent bond, they exist in the solid phase carrier surface stably as compared with the case where it is based on the Pori cation.
[0009] As a strange method of the method of using the electric charge of DNA, the suspension of the PCR product embellished with the amino group is carried out to SSC (standard salt citrate buffer solution). Point arrival on the slide glass surface which silanized this After incubating by carrying out, The method of performing the processing and heat-treatment by sodium borohydride in order is reported (Schena, M.et al., Proc.Natl.Acad.Sci.USA93, 10614-10619 (1996)). However, there is a problem that stability not necessarily sufficient by this fixed method is hard to be acquired. With DNA chip technology, a detection limit becomes important. Therefore, development of the technology which fixes a DNA fragment stably in sufficient quantity for the solid phase carrier surface contributes to improvement in the detection limit of hybridization with the sample nucleic acid fragment which carried out the sign to the fixed DNA fragment greatly.
[0010] (2) when the DNA fragment to fix is an synthetic oligonucleotide Compound the oligonucleotide which introduced the labile machine and point arrival of this oligonucleotide is carried out to the solid phase carrier surface which carried out the surface treatment. a covalent bond is carried out ("protein, nucleic acid and enzyme", 43 volumes, and (1998) --) 2004-2011, Lamture, J.B.et al., Nucl.Acids Res., 22, 2121-2125, 1994 and Guo.Z., et al., Nucl.Acids Res., 22, 5456-5465, 1994. For example, the method to which an amino group introduction oligonucleotide is made to react, and the method of making an aldehyde group introduction oligonucleotide react to this slide glass are known under PDC (p-Feni range isothiocyanate) existence by the slide glass which introduced the amino group. These two methods are fixed stably [an oligonucleotide] on the solid phase carrier surface compared with the method of using the electric charge of DNA of the above (1). [however, the method of the method in which PDC is made to exist having the slow reaction of PDC and an amino group introduction oligonucleotide, and using an aldehyde group introduction oligonucleotide] If it has the problem that the stability of Schiff base which is a resultant is low (hydrolysis usually takes place easily) and the strong functional group of an interaction with DNA exists in a solid phase surface like an amino group further on the whole surface Since the nucleic acid fragment which is analyte adheres easily nonspecific all over a DNA chip, there is a problem of blocking detection. For this reason, in order to prevent this, the process of blocking which closes an unreacted functional group was required.
[0011]
[Problem to be solved by the invention] This invention can combine with the solid phase carrier surface the DNA fragment prepared independently beforehand by a quick reaction. And let it be the technical problem to offer the fixed method with a resultant able to maintain combination stably, the DNA chip which does not

need a blocking process in particular, and the detection method of a nucleic acid fragment.

[0012]

[Means for solving problem] The above-mentioned technical problem was solved by following this invention.

[0013] (1) The fixed method to the solid phase carrier of the DNA fragment characterized by forming a covalent bond between this compound and a DNA fragment by performing optical radiation, contacting a DNA fragment to the solid phase carrier which the compound in which activity is shown by optical radiation has combined with the surface in the liquid phase. In the fixed method of this invention, it is desirable that the compound in which activity is shown by optical radiation is a compound which has nitrene, carbene, a radical, a carbon electrophilic agent, a diazonium machine, an azido group, a JIAJIRIN ring, or a diazo group.

(2) The DNA chip obtained by the above-mentioned method.

[0014] (3) The process which gives the water liquid containing the nucleic acid fragment sample which carried out the sign with the fluorescent substance or the radioactive substance to the surface of the above-mentioned DNA chip, The process which fixes the DNA fragment currently fixed to the DNA chip, and the nucleic acid fragment sample which has complementation on a DNA chip by hybridization, And the detection method of a nucleic acid fragment which consists of a process which detects fluorescent labeling or the radioactive sign of a sign nucleic acid fragment sample fixed on the DNA chip of having complementation to the DNA fragment on a DNA chip.

(4) The process which gives the water liquid containing the intercalator which has a fluorescence generating machine or a conductive machine, and a nucleic acid fragment sample to the surface of the above-mentioned DNA chip, The process which fixes the DNA fragment currently fixed to the DNA chip, and the nucleic acid fragment sample which has complementation on a DNA chip by high BURIDAZEISHON, And consist of a process which detects the electric current which flows through the fluorescence or the conductive machine generated from the fluorescence generating machine of the intercalator incorporated in the hybrid construction formed from the DNA fragment and nucleic acid fragment sample of the DNA chip. The detection method of a nucleic acid fragment of having complementation to the DNA fragment on a DNA chip.

[0015] The desirable mode of the fixed method on the surface of a solid phase carrier of the DNA fragment of this invention is as follows.